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Production of Cytokines in Patients Infected by Hepatitis C Virus

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T helper type 1 (Th1) cytokines play an important role in antiviral defence. The purpose of this study was to quantify by ELISA IL2, soluble receptor of IL2 (IL2Rs), IFNγ TNFβ, IL4, IL6 and IL10 levels in the sera of 134 HCV-positive patients, 69 of whom were coinfected with HIV, and in 54 HIV-HCV-negative patients. The mean IL2Rs and IFN serum levels were much higher in patients with anti-HCV than in the control group, whereas the mean IL4 and IL6 levels were lower in patients infected with HCV. There were no significant differences in cytokine levels between patients with and without HIV. There were significantly less patients with HCV than controls with IFN γ levels under cut-off, and significantly more patients with HCV with IL4 levels under cut-off. Although serum level of cytokines must be interpreted with caution, the results suggest that Th1 response is enhanced in HCV infection. ***J. Med. Virol. 55:89–91, 1998.***

KEY WORDS: HIV; HCV; Th1; Th2; cytokines

INTRODUCTION

T helper type 1 (Th1)-like and T helper type 2 (Th2)-like cells are involved in the response to various infectious antigens (Del Prete et al., 1994). Viruses are more likely to induce a Th1 response (Ada and Blanden, 1994), Th1 cytokines playing an important role in the anti-viral defence by activating T cells and phagocytes. Little is known on the role of cytokines in the course of hepatitis C virus (HCV) infection. We measured therefore the serum levels of various Th1 and Th2 cytokines in a group of 134 patients with HCV who were compared to 54 control patients.

PATIENTS AND METHODS

From a group of 150 patients investigated previously with HCV (Cribier et al., 1995a), the sera of 134 patients could be used for the dosage of cytokines. Sixty-nine of the 134 patients were coinfected by HIV (CDC group A: $n = 28$, group B: $n = 9$, group C: $n = 32$, mean CD4 count $293 \pm 174 \times 10^6$/l; range 10–870). HCV positivity was established by two commercial third generation ELISA (Ortho HCV 3.0™, Ortho Diagnostic Systems Rarital, New Jersey, USA and Murex anti-HCV™, Murex Diagnostics, Dartford, England UK), as required by French law. As a control group, the sera of 54 HCV-negative and HIV-negative patients was tested.

IL2, IL2Rs, IFN, IL4, IL6 and IL10 serum level were measured by ELISA (Kit Predicta™, Genzyme, Cambridge, Massachussets, USA). Conventional ELISAs were carried out using 100 μl of serum according to the manufacturer's instructions and the results were quantified by a microplate reader at 450 nm (Diagnostics Pasteur LP 400).

HCV RNA was detected in the sera by classical reverse transcription followed by a nested polymerase chain reaction (RT-PCR), as described previously (Cribier et al., 1995b). Quantification of HCV RNA was carried out using the branched DNA technique (Quantiplex HCV RNA 1.0™, Chiron) on 50 μl serum samples, according to the manufacturer's instructions.

RESULTS

The patients were 94 males and 40 females of 37 ± 11.2 years of age, 69 were infected with HIV and HCV and 65 were not infected. Twenty seven patients had ALT levels greater than 1.5 × the upper normal limit. HCV RNA was detected by RT-PCR in 117, and the HCV RNA concentration was quantifiable in 84 patients by the bDNA technique. The mean HCV RNA level was $66 \pm 78 \times 10^5$ Eq genome/ml. Liver biopsies were not available, and the approximate duration of HCV infection could not be reliably established. The clinical data and differences between HIV positive and

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TABLE I. Mean Cytokine Levels ± SD Determined by ELISA (Genzyme) in Sera of HCV Infected Patients and in HCV(–) HIV(–) Patients

	IL2 (pg/ml)	IL2Rs (pg/ml)	IFNγ (pg/ml)	IL4 (pg/ml)	IL6 (pg/ml)	IL10 (pg/ml)
All HCV+ patients (n = 134)	13.1 ± 81	2575 ± 1588	13 ± 16.5	7.1 ± 17.7	29.1 ± 64	10.9 ± 10.1
HCV(+) HIV(–) (n = 65)	9.4 ± 45	2268 ± 1332	12.5 ± 17	9.6 ± 24	44.8 ± 85	11.4 ± 12
HCV(+) HIV(+) (n = 69)	16.8 ± 105	2864 ± 1756	13.4 ± 16	4.8 ± 8.3	15.4 ± 34	12.4 ± 9.8
HCV(–) patients (n = 54)	4.2 ± 7.1	406 ± 292	1.7 ± 3.2	19.7 ± 30.6	56.7 ± 85	9.2 ± 6.4
Comparison HCV(+) vs HCV(–)	P = ns	$P < 10^{-9}$	$P < 10^{-9}$	$P < 10^{-2}$	$P < 0.05$	P = ns

HIV negative patients were highlighted in our previous report (Cribier et al., 1995a).

Cytokine Levels

The mean levels of IL2, IL2Rs, IFN, IL4, IL6 and IL10 in the patients with HCV and in the control group are detailed in Table I. Since attributing an "0" value to cytokine levels that were under the cut-off for ELISA could be considered as a bias, we have calculated the number of patients with values under cut-off in each group and the mean cytokine levels of patients having positive cytokine levels. IL2Rs levels were strongly positive in all cases.

- IL2 was under cut-off in 64.7% of controls, vs. 72.8% in patients with HCV (P = ns). The mean IL2 level was 11.4 ± 7.6 pg/ml in controls, vs. 43 ± 145 pg/ml in the HCV positive group (P = ns).
- IFN was under cut-off in 77.4% of controls vs 28.8% of patients with HCV ($P < 10^{-9}$). Mean level was 18.3 ± 16.9 pg/ml in patients with HCV vs. 7.4 ± 1.8 pg/ml in controls ($P < 10^{-3}$).
- IL4 was under cut-off in 13.7% of controls vs 67.5% of patients with HCV ($P < 10^{-9}$). The mean level was 22.9 ± 30 pg/ml in patients with HCV vs. 22.4 ± 24.7 pg/ml in controls (P = ns).
- IL6 was under cut-off in 43% of controls vs. 68.3% in the HCV positive group ($P < 10^{-2}$). The mean IL6 level was 100 ± 91 pg/ml in controls vs. 91.7 ± 86 pg/ml in patients with HCV.
- IL10 was under cut-off in 49.5% of controls vs. 25.6% of patients with HCV ($P < 10^{-3}$). The mean IL10 level was 13.6 ± 8.2 pg/ml in controls vs. 15.4 ± 8.6 pg/ml in patients with HCV (P = ns).

The mean cytokine levels was analyzed in groups of patients defined by the ALT levels, RT-PCR positivity or HCV RNA levels. The mean IL2Rs level in patients with quantifiable HCV RNA by the bDNA method was 2720 ± 1654, whereas it was 2329 ± 1450 pg/ml (P = ns) in the remaining patients in whom the HCV RNA titre was lower than the cut-off. The coefficient of correlation between IL2RS and HCV RNA levels was not significant (P = ns). In the same way, differences were not found in any cytokine level according to the ALT level (normal ALT vs. elevated ALT levels) or to the presence/absence of HCV RNA detected by RT-PCR.

DISCUSSION

In this study, IL2Rs and IFN levels were strongly increased in patients infected by HCV, whereas IL4 and IL6 levels were significantly lower than in controls. The data obtained by quantifying cytokines in the serum must be interpreted with caution, due to their low and variable production. Nevertheless, our results from a large group of patients suggest an enhanced Th1 response. Surprisingly, there were no significant differences between HIV positive and HIV negative patients, suggesting that the Th1 response observed in patients with HCV persists in case of coinfection by HIV. Increased IL2Rs serum levels were already described in patients with HCV (Makris et al., 1994, Hayashi et al., 1995, Morishima et al., 1995). Makris et al. (1994) found a correlation between the severity of HCV induced hepatic changes and the levels of IL2Rs, and both Hayashi et al. (1995) and Morishima et al. (1995) showed a decrease in IL2Rs levels after interferon treatment. We have not found any correlation between HCV RNA levels and cytokine serum levels. Similarly, Dumoulin et al. (1997) could not find any significant correlation between RNAm levels of IL10, IFNγor IL2 and patient characteristics, ALT levels or virus load. It is therefore likely that enhancement of cytokine production is not related to the replication of the virus itself, but rather to the hepatic changes due to viral infection. Actually, Morshed et al (1993) showed that IFN and the IL2Rs gene were upregulated in the liver in case of HCV infection and Napoli et al. (1996) demonstrated that IFN and IL2 RNAm expression was correlated with histological fibrosis and portal inflammation. It is not known whether these cytokines are only markers or if they could be involved in the progression of the disease.

Our results contrast with those published by Cacciarelli et al. (1996), who found very high serum levels of IL4 and IL10 in patients with HCV but also increased IFNγ and IL2 levels. Both studies are nevertheless suggestive of an activated T-cell response, but it remains to be established whether the Th1-Th2 cytokine levels are of clinical importance. Tsia et al. (1997) suggested that activation of Th2 responses in the acute stage of hepatitis C may play a role in the development of chronicity. Lechman et al. (1996) have also shown that a T-helper activation could be of major importance for the prognosis of HCV infection. Further in vitro studies are needed to explain the possible role of cytokines in viral replication.

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